Fly Head Sections Using Martin Heisenberg Fly Collars

Leslie Vosshall, Rockefeller University, 10/13/2002

Information can also be found on the Fischbach Lab Home Page at the following links.

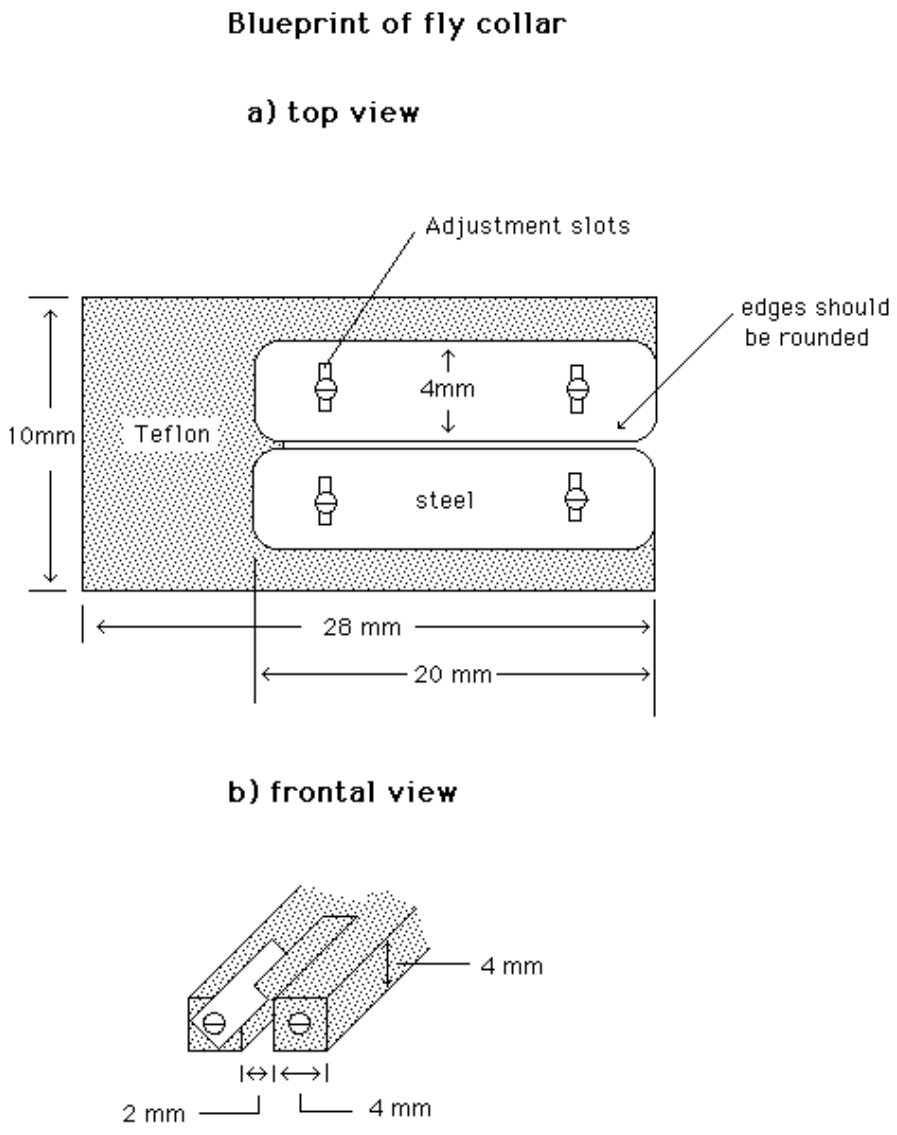
Technique in German:
http://brain.biologie.uni-freiburg.de/text/Methoden/Kragentechnik.html

Examples of fly head sections obtained from paraffin-embedded tissue:
http://brain.biologie.uni-freiburg.de/text/paraffinFi.html

1. Anesthetize flies using carbon dioxide or hypothermia on wet ice.
2. Use forceps to pick up individual flies by grabbing the proximal wing joint. Thread the fly into the collar by first "hanging" fly at the mouth of the collar, with head on top of the blades and body below the blades.
3. Using the tips of the forceps tightly clamped together, gently nudge the fly into the collar by pushing the forcep tips against the neck of the fly. Be careful to be gentle, otherwise you will damage the fly or decapitate it. If decapitation occurs, gently remove head and push body down to avoid interfering with the threading of subsequent flies. It does not affect the quality of the results if the legs of the flies are pointing up through the blades.
4. Best results are obtained if you load each collar with no more than 12 flies. We typically do 6 females and then 6 males, to ensure equal representation of both sexes.
5. When collar is fully loaded, cover the heads with a thin stripe of Tissue Tek OCT (available from VWR Scientific). Fill a VWR plastic casting tray with OCT and quickly invert collar into tray. Immediately place tray/collar onto a metal rack suspended in an ethanol/dry ice bath. Be careful to keep ethanol from contacting the OCT, as this will prevent it from freezing solid and will make sectioning nearly impossible.
6. When samples are fully frozen, the OCT will be pure white.
7. Using a paper towel, carefully pry frozen sample from casting tray, being careful to avoid any contact of ethanol with the sample.
8. Using a clean, sharp razor blade, carefully trim the block to expose the sides of the collar. It is useful to use a blunt tool (such as the metal blocks supplied with bench top heating blocks) to apply a downward force onto the razor blade.
9. Once block has been reduced to the collar and a thin strip of OCT covering the fly heads, carefully use a clean razor blade to pry the fly sample from the Teflon collar. Immediately place collar on dry ice, as it is very liable to thaw at room temperature.
10. Working rapidly, trim sample into a flattened pyramid with no more than one fly head diameter of OCT remaining on all sides of the strip of fly heads. The wider base of the pyramid will be glued to the sample holder with OCT. The narrower top of the pyramid will contain your 12 fly heads, carefully trimmed to display parallel surfaces. Samples can be stored at -80^{o}C at this point for up to 1 week for RNA in situ hybridization and 1 month for antibody staining. Store trimmed blocks in a 15 ml polypropylene screw-cap tube to avoid deterioration due to exposure to air ("freezer burn"). Allow samples to warm to temperature of cryostat before attempting to section.

11. Attach block to a metal sample holder using more OCT. Ensure that sample is securely fastened, or it will fall off during sectioning.
12. Collect 10-20 µm (14 µm is typical) cryostat sections and continue with protocol as desired.

Plans for manufacturing the fly collar:
http://brain.biologie.uni-freiburg.de/pics/atlas/collar.gif



We have ours manufactured by the Rockefeller University machine shop. It is crucial that the steel blades be smooth, non-jagged, and not sharp, or all flies will be decapitated upon threading. The blades should also be adjustable in width, to accommodate variation in the sizes of fly heads. A collar with too-wide spacing will cause animals to fall between the blades; too-narrow spacing will make it difficult to thread most flies efficiently. We have saved money by dispensing with the metal "gate" indicated in the frontal view. This is meant to prevent the escaping of flies, which we have not found to be a serious issue, provided that the CO2 anesthesia is sufficiently deep.

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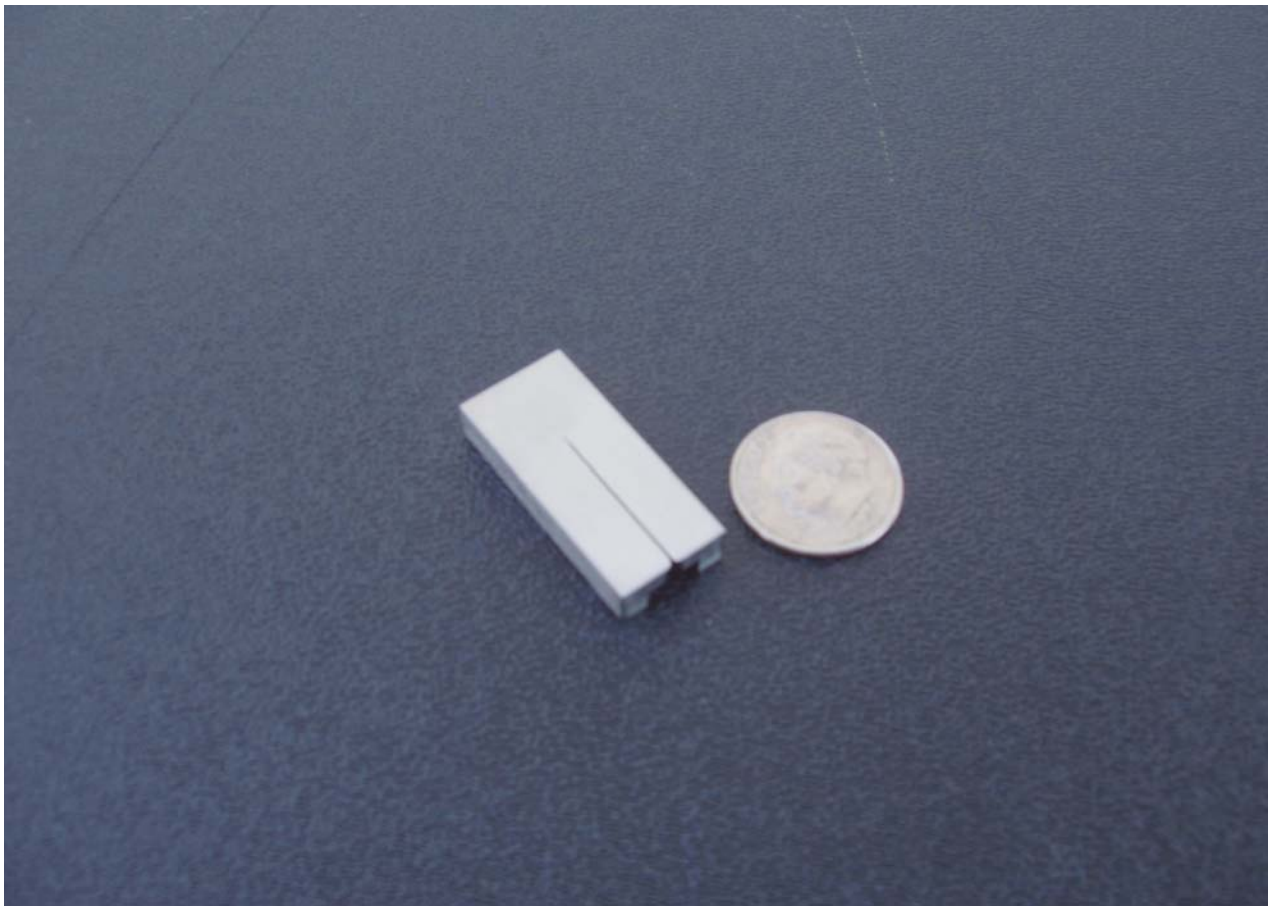

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